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Biological Bases for Cancer Dose-Response Extrapolation Procedures

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The Moolgavkar-Knudson theory of carcinogenesis of 1981 incorporates the viable portions of earlier multistage theories and provides the basis for both the linearized multistage and biologically based dose-response extrapolation methodologies. This theory begins with the premise that cancer occurs because irreversible genetic changes (mutations) are required for transformation of normal cells to cancer cells; incidence data are consistent with only two critical changes being required, but a small contribution from three or higher mutation pathways cannot be ruled out. Events or agents that increase the rate of cell division also increase the probability that one of these critical mutations will occur by reducing the time available for repair of DNA lesions before mitosis. The DNA lesions can occur from background causes or from treatment with mutagenic agents. Thus, the equations describing incidence as a function of exposure to carcinogenic agents include two separate terms, one accounting for mutagenic and one for mitogenic stimuli. At high exposures these interact, producing synergism and high incidence rates, but at low exposures they are effectively independent. The multistage models that are now used include only terms corresponding to the mutagenic stimuli and thus fail to adequately describe incidence at high dose rates. Biologically based models attempt to include mitogenic effects, as well; they are usually limited by data availability.

Introduction

Amid the controversy that has swirled around procedures for extrapolating cancer hazard functions to very small exposures, there has been a frequent assertion that the procedures have no scientific foundation. Implied in that assertion is the idea that procedures lacking such a foundation cannot serve a useful function in public health. This paper explores the foundations of dose-response models.

A variety of mathematical models have been used (or proposed to be used) over the last 15 years to draw inferences about the hazard function for cancer in exposures far below where a dose-response can be observed. They can be divided into two groups: those based on some tolerance distribution (probit, logit, Weibull) and those that derive, one way or another, from models for the age-specific incidence of cancer. Included among the latter are the gamma-multihit procedure, the linearized (LMS) procedure of Guess et al. (*1*), and the several recent procedures sometimes called biologically based models (*2–4*).

"Biologically based models" carries a connotation that the widely used probit, Weibull, and LMS procedures are not based on the biology we know. That clearly is not so. What is true is that the more recent procedures are derived from a more nearly complete theory—Moolgavkar and Knudson's two-event theory of carcinogenesis (*5*)—and from a richer observational base. Thus, they are more likely to be reliable outside the observable range.

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Tolerance-Distribution Models

It is now generally accepted that the probit and Weibull models and related procedures are phenomenologically based. They successfully describe a broad range of phenomena relevant to the estimation of a carcinogen hazard function. Probit and related mathematical models describe the dose-response curves usually observed in the medical sciences, whereas Weibull-type functions describe mortality. Yet, it is also recognized that there are not underlying theoretical bases for these particular functions. Further, the data used to derive these models are not precise enough to permit good tests of their reliability, even at incidences in the 1% range (W. J. Adams et al., unpublished results). Thus, no statement can be made about how well each might represent reality when extrapolated to incidences well below the observable range (i.e., risks of 10^{-3} or smaller). Because the incidence predicted by the probit function declines more rapidly with decreasing dose than that of any other commonly used function, it might be considered to represent a conceptual (not statistical) lower bound to the extrapolated function. Such use is arbitrary.

Model-Free Procedures

Gaylor et al. (*6*) and Krewski et al. (*7*) have described very similar procedures for setting exposure limits based on bioassay data. The basis for these appears to be entirely phenomenological, although some theory must underlie their use (if only the theory that response is some monotonically increasing function of exposure). We would assert that the

justification for choosing the linear-through-zero approach follows from the same biological theory that inspired the LMS procedure (*vide infra*). However, to the extent that these procedures are believed to have no basis in theory, their use is arbitrary.

Multi-Event Models and Procedures

The family of multi-event models includes a variety of standard-setting procedures and mathematical models, including the LMS and gamma-multihit procedures, the Armitage-Doll model (8), and models derived by Moolgavkar and Knudson (*5*) and others (*2–4,9*). These have a long and honorable history [described some time ago by Whittemore and Keller (*10*)]. The formulation was developed to describe the age-specific incidence of adult cancers. If it is assumed that carcinogens behave as chemical electrophiles, undergoing chemically first-order reactions with DNA, then the Guess et al. exponential formulation in dose (*1*) falls out naturally. However, out Moolgavkar (*11*) pointed in 1979 that the Armitage-Doll model does not fit the age-specific incidence data for most cancers, particularly those of the sex organs and of childhood. If the validity of the Guess et al. (*1*) formulation depended on the general validity of the Armitage-Doll model, we would have to conclude that the use of these models should be abandoned. However, that model can equally well be derived from the more general Moolgavkar description of the incidence by making certain assumptions. Thus, these procedures can be valid under a certain set of conditions. What follows is a brief description of the theory of carcinogenesis as it now stands, leading to a discussion of the limitations of these simplified procedures and the possible strengths and limitations of more complex models based on the Moolgavkar-Knudson theory.

We will not discuss further the gamma-multihit model. Its theoretical base is identical to that of the other multi-event models. What distinguishes the gamma-multihit model is the assumption of a particular function for the dose response. Apparently, this form was assumed for the model's tractability, since the resulting equations can be solved exactly. There seems to be no reason to believe that these equations adequately represent reality.

Theory of Carcinogenesis

Cancer is now regarded as a disease of differentiation, by which is meant that the cells that grow into cancer divide when they should undergo differentiation. Genetic change (alterations heritable at the level of the cell) is required, but epigenetic risk factors clearly are important as well. The chalones discussed before 1980 (*13*) now are identified as the protein hormones called "growth factors" (*6,14*). The critical genetic changes somehow involve these growth factors. Yet, the fundamental concepts that are the modern equivalents of growth factors go back half a century of more.

In 1981, Moolgavkar and Knudson (*5*) described the first synthesis of old concepts that provides a satisfactory theory of carcinogenesis. A similar theory was described shortly thereafter by Greenfield et al. (*2*). The theory is built on two fundamental concepts: Mutations occur when unrepaired DNA lesions are present at mitosis, and at least two mutations in critical genetic loci are required to convert a phenotypically normal cell to a cancerous cell.

This theory assumes that cancers are clonal, that they do not ordinarily start in tissues that do not include cells capable of further division, and that mutant (cancerous) cells can continue to mutate and thus evolve both genotypically and phenotypically. This evolution confers survival advantages on the daughter clones; it provides at the cellular level for the phenomenon called "progression" by pathologists (*15*). Typically this progression ends with the appearance of a clone capable of rampant metastatic growth and the death of the host. Much has been learned in the past decade about the nature of the critical loci that are altered to bring about cancer. These alterations affect the system by which intercellular regulation of mitosis and differentiation takes place. This knowledge illuminates and solidifies the basic theory propounded by Moolgavkar and Knudson. Several mathematical formulations of this theory have appeared (*4,9,16,17*). A complete solution has been published that recognizes that each of the processes is stochastic (*12,16*).

The biological model encoded by these mathematical formulations can be described as follows (*2–5,15,18,19*): In a tissue containing cells capable of division (stem cells), normal cells can either divide, giving two normal stem cells, or differentiate, giving a cell not capable of further division. If a normal cell enters mitosis with an unrepaired lesion at some critical site, division will yield one normal cell and one mutant cell. Such mutant cells are identified as initiated, borrowing terminology from experimental carcinogenesis. Recent research suggests that these initiated cells respond differently from normal cells to the hormones that affect intercellular growth/differentiation regulation, making them more likely to divide and less likely to differentiate at any hormone concentration.

Initiated cells otherwise behave similarly to normal cells, dividing to give additional initiated cells, differentiating, or dividing with another unrepaired lesion to yield a twice-mutant cell, now (probably) phenotypically cancerous. (Note that this model describes the most frequently followed pathway between normal cells and cancer. It implicitly includes the possibility that more than two mutations can occur before the cancerous phenotype emerges. Since mutation is a rare event, however, such pathways will be rarely observed, just because of the concatenation of low probabilities.)

In Moolgavkar's original formulation (*15,16*), division, differentiation, and mutation were taken as deterministic and assigned rates α, β, and μ, respectively. Normal cells divide at rate α_1, differentiate at rate β_1,and mutate at rate μ_1; initiated cells divide at rate α_2, etc. Now it is recognized that all these are stochastic processes, and the labels are assigned to the several corresponding transition probabilities (*2,3,12,16*).

This theory recognizes that certain treatments may increase the net birth rates of initiated cells. These lead to expansion of the clone of initiated cells; again borrowing from experimental cancer, the process is termed "promotion" (*5,15*). Because it is recognized that adventitious (background or spontaneous) initiation occurs through the natural mutagenic flux, the theory predicts that pure promoters (agents with little or no mutagenic activity) will act as complete carcinogens in ordinary bioassays. This suggests that the term "complete carcinogen" conveys nothing and that its use should be discontinued.

The theory of carcinogenesis rationalizes a large number of observations from experimental science, epidemiology, and clinical practice (*2–5,15*). In addition, a number of predictions made from the theory have been verified, particularly the phenomenon called I-P-I (initiation-promotion-initiation) (*13*). The theory provides a good explanation for the age-specific incidences of essentially all cancers (*5,11*), including the adult cancer for which the Armitage-Doll model provided an earlier, different explanation. The Armitage-Doll explanation is now regarded as inadequate (*20*). Like Armitage-Doll, the current theory is a member of the set of multi-event models, although more specific than earlier ones in that it gives specific identification to the several events. The theory differs from earlier theories in the explicit place provided for effect of increased mitotic rate on cancer risk. Both the various linear extrapolation procedures and the more recent biologically based models can be derived from this theory.

Extrapolation Procedures: Linear Models

It is convenient to discuss derivation of the various procedures, their applicability, and their limitations in the context of Moolgavkar's approximate solution to the age-specific incidence (*5,16*).

$$I(t) \approx \mu_1\mu_2 \int_0^t X(s)\, e^{(\alpha_2-\beta_2)(t-s)}ds \tag{1}$$

Here $X(s)$ denotes the number of normal stem cells at time s, μ_1 and μ_2 are the mutation rates of normal and initiated cells, and $(\alpha_2 - \beta_2)$ is the net birth rate of initiated cells. [As Moolgavkar and Dewanji pointed out (*17*), this equation is valid only for $I(t) < 0.2$; at higher incidence a more nearly exact formulation must be used.] In this formulation, the synergistic effects of initiated cell mitotic rate on mutation probability become apparent.

Eq. (1) assumes only adventitious initiation and no effect of treatment on any of the other parameters. Generalizing, taking $\mu_i = \mu_0 + \mu_i(d)$, where μ_0 is the adventitious rate, we obtain:

$$I(d,t) \approx [\mu_0+\mu_1(d)][\mu_0+\mu_2(d)] \int_0^t X(d,s)\, e^{[\alpha_2(d)-\beta_2(d)](t-s)}ds \tag{2}$$

In other words, treatment is allowed to affect any or all of the several parameters in the model.

If experimental conditions are such that we can assume the integral term to be constant over a series of experiments carried to constant time t, then Eq. (3) results.

$$I(d) \approx [\mu_0+\mu_1(d)][\mu_0+\mu_2(d)] \tag{3}$$

If we further assume $\mu(d)$ to be linear, as Guess et al. did (*1*), we obtain:

$$I(d) \approx \mu_0^2 + (\mu_1 + \mu_2)d + \mu_1\mu_2 d^2 \tag{4}$$

Eq. (4) is the same as the approximate solution to the multistage model for $d << 1$ given by Guess et al. (*1*); it also could provide a rationale for procedures proposed by Gaylor et al. (*6*) and by Krewski et al. (*7*).

Limitations of Linear Models

Two serious constraints exist for the linear models. The approximation from which they can be derived is valid only for relatively low values of I, and there must be no significant increase in the mitotic rate of either normal or initiated cells. In fact, it appears from the recent work of Cohen and Ellwein (*2,3*) that the first condition will usually be met if the second one is met. Their work suggests that treatment conditions that increase mitotic rate cause the incidence versus exposure curve to bend sharply upward. At present, we know only one example of an experiment yielding a tumor incidence in the 20% to 30% range where the dose response is linear, *viz.*, the liver tumors in mice treated with 2-AAF in the ED_{01} study (*21*). According to Cohen and Ellwein's recent analysis (*21*), no evidence for a mitotic rate increase is seen. Otherwise, so far, whenever relevant evidence is available, high incidence seems to be accompanied by mitotic rate increase (*15*). (Note that only a few well-characterized examples are yet available.)

The importance of this mitotic rate increase is quite clear. Application of linear models to high dose-rate data giving high incidence is not appropriate. To apply linear models under those circumstances will greatly exaggerate the estimated incidence. It is especially inappropriate to apply these procedures with data sets from treatment with nongenotoxic compounds. However, it may be appropriate to use these methods for strongly genotoxic compounds, when the data show no evidence for curvalinearity. It is also clear that we need to investigate further the behavior of mitotic rate under treatment with mitogens so that we can begin to address the deficiencies in these linear methods.

Biologically Based Models

Thorslund et al. (*4*) coined the phrase "biologically based models" to describe the family of models they have explored. Implied by the phrase is a notion that other models are not biologically based and thus somehow inferior. This is not necessarily the the case. Thorslund's model structures, in particular, suffer from an inability to incorporate time-dependence of cell number, mitotic rate, etc. They are thus vulnerable to being criticized as unrealistic. Moolgavkar has recently published a series of papers describing increasingly less approximate solutions to the general model. The latest of these gives an exact, though very complex, solution and an application to radiation-induced cancer data (*16*). In principle, these sets of equations should yield reliable estimates of the hazard function below the observable range, since their biological base is the best known. In practice, the successful use of these equations for this purpose will be some time in the future because their solution requires data not generally available, and the effects of various approximations and default parameters have not yet been adequately explored. Nevertheless, this development is one of the most exciting in risk assessment in recent years.

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REFERENCES

1. Guess, H. A., Crump, K. S., and Peto, R. Uncertainty estimate for low-exposure extrapolations of animal carcinogenicity data. Cancer Res. 37: 3475–3488 (1977).
2. Greenfield, R. E., Ellwein, L. B., and Cohen, S. M. A general theory of carcinogenesis. Carcinogenesis 5: 437–445 (1984).
3. Ellwein, L. B., and Cohen, S. M. Comparative analyses of the timing and magnitude of genotoxic and nongenotoxic cellular effects in urinary bladder carcinogenesis. In: Biologically Based Methods for Cancer Risk Assessment (C. Travis, Ed.), Plenum Press, New York, 1989, pp. 181–192.
4. Thorslund, T. W., Brown, C. C., and Charnley, G. Biologically motivated model for cancer risk models. Risk Anal. 7: 109–119 (1987).
5. Moolgavkar, S. H., and Knudson, A. G. Mutation and cancer: a model for human carcinogenesis. J. Natl. Cancer Inst. 66: 1037–1052 (1981).
6. Gaylor, D. W., Chen, J. J., and Kodell, R. L. Experimental design of bioassay for screening and low-dose extrapolation. Risk Anal. 5: 9–16 (1985).
7. Krewski, D., Gaylor, D. W., Bickis, M., and Szyszkowics, M. A model-free approach to low-dose extrapolation. Environ. Health Perspect. 90: 279–285 (1991).
8. Armitage, P., Doll, R. The age distribution of cancer and a multi-stage theory of carcinogenesis. Br. J. Cancer. 8: 1–12 (1954).
9. Mauskopf, J. Paper presented at the 1986 Annual Meeting of the Society for Risk Analysis, Boston, MA.
10. Whittemore, A., and Keller, J. B. Quantitative theories of carcinogenesis. SIAM Rev. 20: 1–30 (1978).
11. Moolgavkar, S. H., and Venzon, D. J. Two-event models for carcinogenesis. Math. Biosci. 47: 55–77 (1979).
12. Moolgavkar, S. H., Cross, F. T., Luebeck, G., and Dagle, D. E. A two-mutation model for radon-induced lung tumors in rats. Radiat. Res. 121: 28–37 (1990).
13. Potter, V. R. A new protocol and its rationale for the study of initiation and promotion of carcinogenesis in rat liver. Carcinogenesis 2: 1375–1379 (1981).
14. Bradshaw, R. A., and Prentis, S., Eds. Oncogenes and Growth Factors. Elsevier Publishing Co., Amsterdam, 1987.
15. Wilson, J. D. Assessment of low-exposure risk from carcinogens: implications of the Knudson-Moolgavkar two-critical mutation theory. In: Biologically Based Methods for Cancer Risk Assessment (C. Travis, Ed.), Plenum Press, New York, 1989, pp. 275–287.
16. Dewanji, A., and Moolgavkar, S. H. A stochastic two-stage model for cancer risk assessment. Part II. The number and size of pre-malignant clones. Risk Anal. 9: 179–187 (1989).
17. Moolgavkar, S. H., and Dewanji, A. Biologically-based models for cancer risk assessment: a cautionary note. Risk Anal. 8: 5–7 (1987).
18. Moolgavkar, S. Carcinogenesis modeling: from molecular biology to epidemiology. Annu. Rev. Public Health 7: 151–169 (1986).
19. Knudson, A. G. A two-mutation model for human cancer. Adv. Viral Oncol. 7: 1–17 (1987).
20. Moolgavkar, S. H. The Multi-stage theory of carcinogenesis and the age distribution of cancer in man. J. Natl. Cancer Inst. 61: 49–52 (1979).
21. Cohen, S. M., and Ellwein, L. Proliferative and genotoxic cellular effects in a 2-acetylaminoflourene bladder and liver carcinogenesis: biological modelling of the ED_{01} study. Toxicol. Appl. Pharmacol. 104: 79–93 (1990).